Protocol for the Gibson Assembly

Gibson Assembly Master Mix

5x ISO buffer	400µl
T5 exonuclease (10U/µl)	1µl
Phusion polymerase (2U/µl)	25µl
Taq ligase (40U/µl)	200µl
ddH2O	375µl
Total	1ml

Prepare the Gibson Assembly Master Mix before the reaction. Use designed primers for PCR to get DNA with the overlapping sequence. Gel purify the DNA, and measure the concentration afterwards. Add the linearalized DNA parts to one PCR tube to get a total of 5ml liquot, and make sure each part has the same mole ratio. Add 15ml Gibson Assembly Master Mix into the PCR tube and react for 1 hour at 50℃. Place the PCR tube on ice or store at 4℃ and it can be used for direct transformation of host cells